APPENDIX A

Claim Listing

Claim Listing

That which is claimed is:

1.(Withdrawn) A method to determine the optimal length of an immunobiologically-active liner peptide epitope of a polypeptide, the method comprising the steps of:

a) providing a curve characterizing the hydrophilicity and/or hydrophobicity of the linear sequence of amino acid residues of a polypeptide;

b) generating at least one potential epitope set comprising at least one potential epitope by fitting a window of the curve of step (a) to a mathematically generated continuous curve, the continuous curve having repeating values at regular intervals with at least a maximum positive value, the window containing a specific number of amino acid residues and the window is lagged through the curve of step (a);

c) increasing the number of residues in the window after each lagging;

d) determining and ranking potential epitopes for each set by selecting potential epitopes having a positive-fit correlation value determined by fitting curves in step (b) thereby providing a set of ranked potential epitopes for each window of residues used in step (b), the most positive-fit correlation value ranked first in each potential epitope set;

e) examining the positioning of at least the highest ranked potential epitopes of each set relative to the linear sequence of the plot of step (a) to determine at least one set of potential epitopes that exhibit alternating positioning

about an equilibrium position wherein the ranking values of the potential epitopes coverage towards or diverge away from the equilibrium position; and

f) designating the potential epitopes of the set having the most alternating ranking values that coverage or diverge as the immunologically active epitopes which has an optimal length equating to numeric value of amino acid residues in the potential epitopes.

2. (Withdrawn) The method according to claim 1 wherein the mathematically generated curve is generated by a negative cosine curve function.

3. (Withdrawn) A method to determine the optimal length of an immunobiologically active linear peptide epitope of a polypeptide characterized by a hydrophobic-hydrophilic-hydrophobic motif, the method comprising the steps of:

a) assigning an average hydropathy value to each amino acid of the polypeptide;

b) generating a hydrophilicity plot using the average hydropathy value of each amino acid;

c) fitting the curve segment of the hydrophilicity plot to a negative cosine function, wherein a specific period number values of the negative cosine function equates to the number of amino acids in the curve segment, the period number increasing within a predetermined chosen period number range after each sequential lagging through the hydrophilicity plot thereby providing fit-correlation values for each curve segment across the linear sequence when using the specific period number value:

d) generating a potential Ho-Hi-Ho epitope set for each specific period number value within the chosen period number range, wherein each potential Ho-Hi-Ho epitope set contains potential Ho-Hi-Ho epitopes that have a fit-correlation value;

e) ranking each potential Ho-Hi-Ho epitope in the potential Ho-Hi-Ho epitope set according to positive fit-correlation values wherein the epitope having highest positive-fit correlation value is ranked number one thereby providing ranked Ho-Hi-Ho potential epitopes for each specific period number value;

f) examining the positioning of at least the highest ranked Ho-Hi-Ho potential epitopes of each set relative to the linear sequence of the plot of step (a) to determine at least one set of Ho-Hi-Ho potential epitopes that exhibit alternating positioning about an equilibrium position wherein the ranking value of Ho-Hi-Ho potential epitopes coverage towards or diverge away from the equilibrium position; and

g) designating the Ho-Hi-Ho potential epitopes of the set having the most alternating ranking values that coverage or diverge as the immunologically active epitopes which have an optimal length equating to numeric value of amino acid residues in the potential epitopes.

4. (Withdrawn) The method according to claim 3 wherein said hydrophilicity curve is generated using Kyte-Doolittle hydropathy values with reserved signs.

5. (Withdrawn) The method according to claim 3 further comprising choosing the potential epitope set having the highest fit correlation value found in step (c) if more than one potential epitope set exhibits the same number of alternating ranking as examined by step (f).

6. (Withdrawn) A Ho-Hi-Ho epitope of a polypeptide, said Ho-Hi-Ho epitope characterized by a hydrophobic-hydrophilic-hydrophobic motif having a optimal length of amino acid residues determined by method of claim 3.

7. (Withdrawn) The Ho-Hi-Ho epitope according to claim 6 wherein the amino acid residues are altered by replacing amino acids to increase or decrease the fit correlation between the hydrophilicity curve and the negative cosine curve thereby increasing or decreasing the affinity for the epitope by immune components.

8. (Withdrawn) A method for determining the viability of a protein comprising:

a) finding the immunobiologically active epitopes of a polypeptide and their optimal length according to the method of claim 3; and

b) comparing the optimal length found in step (a) to the optimal length found in antipolypeptide antisera.

9. (Withdrawn) A antisera specific for a Ho-Hi-Ho epitope of contiguous amino acid residues from a polypeptide wherein said epitope is defined by a motif of two hydrophobic and on hydrophilic regions arranged in the following manner

hydrophobic – hydrophilic – hydrophobic

wherein said epitope has an optimal length of amino acid residues determined by method of claim 3.

10. (Withdrawn) An antigenic composition comprising a Ho-Hi-Ho epitope of contiguous amino acid residues from a polypeptide wherein said epitope is characterized by a hydrophobic-hydrophilic-hydrophobic motif having an optimal length of amino acid residues determined by method of claim 3.

11. (Withdrawn) The antigenic composition comprising a nucleic acid molecule coding for a Ho-Hi-Ho epitope of contiguous amino acid residues from a polypeptide wherein said epitope is characterized by a hydrophobic-hydrophilic-hydrophobic motif having an optimal length of amino acid residues determined by method of claim 3.

12. (currently amended) A ~~diagnostic testing~~ method for detecting a protein of known sequence comprising the steps of :

~~(i) providing a sample;~~

~~(ii) contacting said sample with antisera specific for a Ho-Hi-Ho epitope of contiguous amino acid residues from a polypeptide wherein said epitope is characterized by a hydrophobic-hydrophilic-hydrophobic motif having an optimal length of amino acid residues determined by a method comprising the steps of:~~

~~a) assigning an average hydropathy value to each amino acid of a polypeptide;~~

~~b) generating a hydrophilicity plot using the average hydropathy value of each amino acid;~~

~~c) fitting a curve segment of the hydrophilicity plot to a negative cosine function, wherein a specific period number value of the negative cosine function equates to the number of amino acids in the curve segment, the period number increasing within a predetermined chosen period number range after each sequential lagging through~~

~~the hydrophilicity plot thereby providing fit correlation values for each curve segment across the linear sequence when using the specific period number value;~~

~~d) generating a potential Ho Hi Ho epitope set for each specific period number value within the chosen period number value within the chosen period number range, wherein each potential Ho Hi Ho epitope set contains potential Ho Hi Ho epitopes that have a fit correlation value;~~

~~e) ranking each potential Ho Hi Ho epitope in the potential Ho Hi Ho epitope set according to positive fit correlation values wherein the epitope having highest positive fit correlation value is ranked number one thereby providing ranked Ho Hi Ho potential epitopes for each specific period number value;~~

~~f) examining the positioning of at least the highest ranked Ho Hi Ho potential epitopes of each set relative to the linear sequence of the plot of step (a) to determine at least one set of Ho Hi Ho potential epitopes that exhibit alternating positioning about an equilibrium position wherein the ranking values of the Ho Hi Ho potential epitopes coverage towards or diverge away from the equilibrium position; and~~

~~g) designating the Ho Hi Ho potential epitopes of the set having the most alternating ranking values that coverage or diverge as the immunologically active epitopes which have an optimal length equating to numeric value of amino acids residues in the potential epitopes;~~

~~(iii) detecting the binding of said antisera to a polypeptide in said sample.~~

determining an optimal immunobiologically active linear epitope of said protein of known sequence, wherein said protein is comprised of a plurality of amino acids, and wherein said optimal immunobiologically active linear epitope is characterized by a hydrophobic-hydrophilic-

hydrophobic (Ho-Hi-Ho) motif determined by a method comprising the steps of:

assigning an average hydropathy value to each of said plurality of amino acids of said protein of known sequence;

generating a hydrophilicity plot using said average hydropathy value;

fitting each of a plurality of curve segments of said hydrophilicity plot to one of a plurality of a negative cosine functions, wherein one of a plurality of a specific period number values of said plurality of negative cosine functions equates to a particular number of amino acids in one of said plurality of curve segments, said period number value increasing within a predetermined chosen period number range after a sequential lagging of each of said plurality of curve segments through said hydrophilicity plot thereby providing a fit-correlation value for each of said plurality of curve segments across said known sequence of said protein when using one of said plurality of said period number values;

generating a potential Ho-Hi-Ho epitope set for each of said plurality of specific period number values within said chosen period number range, wherein said potential Ho-Hi-Ho epitope set contains at least one potential Ho-Hi-Ho epitope in which said fit-correlation value is positive;

ranking each of said potential Ho-Hi-Ho epitopes of said potential Ho-Hi-Ho epitope set and assigning a ranking value to each of said potential Ho-Hi-Ho epitopes according to said fit-correlation value, wherein said potential Ho-Hi-Ho epitope with a highest positive fit-correlation value is ranked number one, thereby providing a ranked Ho-Hi-Ho potential epitope for each of said plurality of specific period number values;

examining said ranking value of each of said potential Ho-Hi-Ho epitopes relative to said hydrophilicity plot to determine at least one potential Ho-Hi-Ho epitope set that exhibits alternating positioning around an equilibrium position, wherein a plurality of said ranking values of said potential Ho-Hi-Ho epitopes converge towards or diverge away from said equilibrium position; and

designating each of said potential Ho-Hi-Ho epitopes, wherein said ranking values exhibit a most alternating ranking value that converges or diverges from said equilibrium position as said optimal immunobiologically active epitope wherein a numeric value of amino acid in said potential Ho-Hi-Ho epitopes is equal to one of said plurality of specific period number values of said negative cosine function;

synthesizing at least one peptide corresponding to at least one of said optimal immunobiologically active linear epitope;

creating at least one antisera against said synthesized peptides corresponding to at least one of said optimal immunobiologically active linear epitope;

providing a sample to be analyzed for said protein of known sequence;

contacting said sample with said at least one antisera; and

detecting a binding of said antisera to said protein of said sample, thereby indicating presence of said protein in said sample.

13. (Withdrawn) A method to determine the optimal length of an immunobiologically-active linear peptide epitope of a polypeptide, the method comprising the steps of:

a) providing a hydrophilicity and/or hydrophobicity plot generated for an amino acid linear sequence of a polypeptide;

b) fitting the plot of step (a) to a mathematically generated continuous curve thereby generating potential epitope sets which include ranked potential epitopes having a specific number of amino acid residues; and

c) comparing the sets of ranked potential epitopes to other generated data to determine the immunobiologically-active linear peptide epitope and its optimal length.

14. (Withdrawn) The method according to claim 13 wherein the other generated data of step (c) is selected from the group consisting of: comparing magnitude of oscillating behavior, comparing the ranked potential epitopes with other epitopes generated by propensity scales, comparing with a previously generated plot and combinations thereof.

15. (Withdrawn) A method to determine the optimal length of an immunobiologically-active linear peptide epitope of a polypeptide, the method comprising the steps of:

a) fitting a hydrophilicity and/or hydrophobicity plot generated for the amino acid linear sequence of a polypeptide to a mathematically generated continuous curve thereby generating potential epitope sets which include ranked potential epitopes having a specific number of amino acid residues, the mathematically generated curve having at least a maximum positive value;

b) positioning the ranked potential epitopes for each set on the hydrophilicity and/or hydrophobicity plot to determine the oscillating behavior of the numeric value of ranked potential epitopes; and

c) deeming the potential epitopes that exhibit the most alternating positioning about an equilibrium position when juxtaposed on the hydrophilicity and/or hydrophobicity plot as the theoretical epitomes and their optimal length corresponds to the specific number of amino acid residues in the set of ranked potential epitopes.

16. (Withdrawn) The method according to claim 15 wherein said hydrophilicity curve is generated using Kyte-Doolittle hydropathy values and the mathematically generated curve is generated by a negative cosine function having a period number equivalent to the window of residues.

17. (Withdrawn) A Ho-Hi-Ho epitope of a polypeptide, said Ho-Hi-Ho epitope characterized by a hydrophobic-hydrophilic-hydrophobic motif having an optimal length of amino acid residues determined by method of claim 16.

18. (Withdrawn) A antisera specific for a Ho-Hi-Ho epitope of contiguous amino acid residues from a polypeptide wherein said epitope is defined by a motif of two hydrophobic and one hydrophilic regions arranged in the following manner

hydrophobic-hydrophilic-hydrophobic

wherein said epitope has an optimal length of amino acid residues determined by method of claim 15.

19. (currently amended) A ~~diagnostic testing~~ method for detecting a protein of known sequence comprising the steps of:

~~(i) providing a sample~~

~~(ii) contacting said sample with anticora specific for a Ho-Hi-Ho epitope of contiguous amino acid residues from a polypeptide wherein said epitope is characterized by a hydrophobic-hydrophilic-hydrophobic motif having optimal length of amino acid residues determined a method comprising the steps of:~~

determining an optimal immunobiologically active linear peptide epitope of said protein, wherein said protein is comprised of a plurality of amino acids, wherein said optimal immunobiologically active linear peptide epitope is characterized by a Hydrophobic-Hydrophilic-Hydrophobic (Ho-Hi-Ho) motif determined by method comprising the steps of:

~~(a)~~ fitting a hydrophilicity/ ~~and/or~~ hydrophobicity plot generated for ~~the~~ said ~~amino acid linear~~ protein of known sequence ~~of a polypeptide~~ to a mathematically generated continuous curve thereby generating at least one potential Ho-Hi-Ho epitope ~~sets~~ set which ~~include~~ includes at least one ranked potential Ho-Hi-Ho ~~epitopes~~ epitope, wherein one of a plurality of a numeric value is assigned to each of said potential Ho-Hi-Ho epitope set corresponding to a fit-

correlation value of said hydrophilicity/hydrophobicity plot to said mathematically generated continuous curve, wherein said mathematically generated continuous curve has a period equal to a ~~having a specific number of~~ number of amino ~~acid~~ acids ~~residues,~~ ~~the~~ corresponding to the length of said potential Ho-Hi-Ho epitope, said mathematically generated curve having ~~at least~~ a maximum positive value;

~~(b)~~ positioning ~~the~~ said at least one ranked potential Ho-Hi-Ho epitope ~~epitopes for each set~~ on ~~the~~ said hydrophilicity/~~and/or~~ hydrophobicity plot to determine ~~the~~ an oscillating behavior of ~~the~~ said plurality of numeric values of said at least one ranked potential Ho-Hi-Ho epitope ~~epitopes~~; and

~~(c)~~ deeming ~~the~~ each of said plurality of ranked potential Ho-Hi-Ho epitope ~~epitopes~~ that exhibit ~~the~~ a most alternating position about an equilibrium position when juxtaposed on ~~the~~ said hydrophilicity/ ~~and/or~~ hydrophobicity plot as ~~the theoretical epitopes~~ said optimal immunobiologically active linear peptide epitope, wherein said optimal immunobiologically active linear peptide epitope and ~~their~~ its optimal length corresponds to ~~the specific~~ a number of amino ~~acid residues~~ acids in ~~the~~ a set of ranked potential Ho-Hi-Ho epitopes; ~~and~~

~~(iii) detecting the binding of said antisera to a polypeptide in said sample~~

synthesizing at least one peptide corresponding to at least one of said optimal immunobiologically active linear epitope;

creating at least one antisera against said synthesized peptides corresponding to at least one of said optimal immunobiologically active linear epitope;

providing a sample to be analyzed for said protein of known sequence;

contacting said sample with said at least one antisera; and

detecting a binding of said antisera to said protein of said sample, thereby indicating presence of said protein in said sample.

20. (Withdrawn) A antigenic composition comprising a nucleic acid molecule coding for an epitope of contiguous amino acid residues from a polypeptide wherein said epitope has an optimal length of amino acid residues determined by method of claim 15.

21. (new) The method of claim 12, wherein said protein is selected from a group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IFN-α, IFN-β, IFN-65, CD2, CD3,CD4, CD5, CD8, CD11A, CD11b, CD11c, CD16, CD18, CD21, CD28, CD32, CD34, CD35, CD40, CD44, CD45, CD54 CD56, K2, K1, Pβ, Oα, Mα, Mβ2, Mβ1, LMP1, TAP2, LMP7, TAP1, Oβ, IAβ, IAα, IEβ, IEβ2, IEα, CYP21, C4B, CYP21P, C4A, BF, C2, HSP, G7a/b, TNF-α, TNF-β,D,L Qa, T1a, COL11A2, DPβ2, DPα2, DPβ1, DP 1, DN 2, DQ 3, DQ 1, DQ DR , DR , HSP-70, HLA-B, HLA-C, HLA-X, HLA-E, HLA-J, HLA-A, HLA-H, HLA-G, HLA-F, NGF, somatotropin, somatomedins, parathormone, FSH, LH, EGF, TSH, THS-releasing factor, HGH, GRHR, PDGF, IGF-I, IGF-II, TGF- , GM-CSF, M-CSF, G-CSF1, erythropoietin, -IICG, 4-N-acetylgalactosaminyltransferase, GM2, GD2, GD3, MAGE-1, MAGE-2, MAGE-3, MUC-1, MUC-2, MUC-3, MUC-4, MUC-18, ICAM-1, C-CAM, V-CAM, ELAM, NM23, EGFR, E-cadherin, N-CAM, CEA, DCC, PSA, Iler2-neu, UTAA, melanoma antigen p75, K19, HKer 8, pMEL 17, tyrosine related proteins 1 and 2, p97, p53, RB, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, FCC, MCC, ras, myc, neu, raf, erb, src, fms,

jun, trk, ret, gsp, hst, bcl, abil, C1q, C1r, C1s, C4,C2, Factor D, Factor B, properdin, C3, C5, C6, C7, C8, C9, C1Inh, Factor H, C4b-binding protein, DAF, membrane cofactor protein, anaphylatoxin inactivator S protein, HRF,MIRL, CR1, CR2, CR3, CR4, C3a/C4a receptor, HIV (gag, pol, gp41, gp120, vif, tat, rev, nef, vpr, vpu, vpx), HSV (ribonucleotide reductase, -TIF, ICP4, ICP8, ICP35, LAT-related proteins, gB, gC, gD, gE, gI, gJ), influenza (hemagluttinin, neuroaminidase, PB1, PB2, PA, NP, M_1, M_2, NS_1, NS_2) papillomaviruses (E1, E2, E3, E4, E5a, E5b, E6, E7, E8, L1, L2), adenovirus (E1A, E1B, E2, E3, E4, E5, L1, L2, L3, L4, L5), Epstein-Barr Virus (EBNA), Hepatitis B virus, ($gp27^s$, $gp36^s$, $gp42^s$, $p22^c$, pol, x) and nuclear matrix proteins.

22.) (new) The method of claim 19, wherein said protein is selected from a group consisting of IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IFN-α, IFN-β, IFN-65, CD2, CD3,CD4, CD5, CD8, CD11A, CD11b, CD11c, CD16, CD18, CD21, CD28, CD32, CD34, CD35, CD40, CD44, CD45, CD54 CD56, K2, K1, Pβ, Oα, Mα, Mβ2, Mβ1, LMP1, TAP2, LMP7, TAP1, Oβ, IAβ, IAα, IEβ, IEβ2, IEα, CYP21, C4B, CYP21P, C4A, BF, C2, HSP, G7a/b, TNF-α, TNF-β,D,L Qa, T1a, COL11A2, DPβ2, DPα2, DPβ1, DP 1, DN 2, DQ 3, DQ 1, DQ DR , DR , HSP-70, HLA-B, HLA-C, HLA-X, HLA-E, HLA-J, HLA-A, HLA-H, HLA-G, HLA-F, NGF, somatotropin, somatomedins, parathormone, FSH, LH, EGF, TSH, THS-releasing factor, HGH, GRHR, PDGF, IGF-I, IGF-II, TGF- , GM-CSF, M-CSF, G-CSF1, erythropoietin, -IICG, 4-N-acetylgalactosaminyltransferase, GM2, GD2, GD3, MAGE-1, MAGE-2,

MAGE-3, MUC-1, MUC-2, MUC-3, MUC-4, MUC-18, ICAM-1, C-CAM, V-CAM, ELAM, NM23, EGFR, E-cadherin, N-CAM, CEA, DCC, PSA, Iler2-neu, UTAA, melanoma antigen p75, K19, HKer 8, pMEL 17, tyrosine related proteins 1 and 2, p97, p53, RB, APC, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, FCC, MCC, ras, myc, neu, raf, erb, src, fms, jun, trk, ret, gsp, hst, bcl, abil, C1q, C1r, C1s, C4,C2, Factor D, Factor B, properdin, C3, C5, C6, C7, C8, C9, C1Inh, Factor H, C4b-binding protein, DAF, membrane cofactor protein, anaphylatoxin inactivator S protein, HRF,MIRL, CR1, CR2, CR3, CR4, C3a/C4a receptor, HIV (gag, pol, gp41, gp120, vif, tat, rev, nef, vpr, vpu, vpx), HSV (ribonucleotide reductase, -TIF, ICP4, ICP8, ICP35, LAT-related proteins, gB, gC, gD, gE, gI, gJ), influenza (hemagluttinin, neuroaminidase, PB1, PB2, PA, NP, M_1, M_2, NS_1, NS_2) papillomaviruses (E1, E2, E3, E4, E5a, E5b, E6, E7, E8, L1, L2), adenovirus (E1A, E1B, E2, E3, E4, E5, L1, L2, L3, L4, L5), Epstein-Barr Virus (EBNA), Hepatitis B virus, ($gp27^a$, $gp36^s$, $gp42^s$, $p22^c$, pol, x) and nuclear matrix proteins.

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Invention: **IMPROVED METHOD OF IDENTIFYING AND LOCATING IMMUNOBIOLOGICALLY-ACTIVE LINEAR PEPTIDES**

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